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MEMBRANES OF *TETRAHYMENA*

III. THE EFFECT OF TEMPERATURE ON MEMBRANE CORE STRUCTURES AND FATTY ACID COMPOSITION OF *TETRAHYMENA* CELLS

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SUMMARY

Membrane core structures as revealed by the freeze-etch electron microscopy and the fatty acid composition measured by gas–liquid chromatography have been analyzed in *Tetrahymena* cells exposed to low temperature for varying periods.

When cells were grown to mid-log phase at the optimal growth temperature of 28 °C and then chilled to 10 °C, cell division was inhibited. However, within 16 h the cells adapted to the low temperature.

Chilling effected drastic structural alterations in the cores of different membrane types (membranes of the pellicula, the alveolar sacs, the endoplasmic reticulum and the nuclei). In all cases, there was a segregation of smooth faces from particle-rich faces in the fracture planes. However, the native membrane state, *i.e.* like that of cells grown at 28 °C, reappeared when the cells adapted to the low temperature.

The total lipids of *Tetrahymena* cells contained primarily even-numbered fatty acids ranging from C_{12} to C_{18}, but we also detected appreciable amounts of C_{20} acids; this has not been reported before. During the initial phase of chilling, when cell division is inhibited, about 50% of the saturated fatty acids were replaced by unsaturated fatty acids, primarily monoenoic, dienoic and trienoic acids.

We conclude that the structural recovery of the membranes in chilled *Tetrahymena* cells is accomplished by a desaturation of membrane fatty acids. This is discussed with respect to membrane "fluidity".

INTRODUCTION

Specific membrane functions such as permeability, excitability and transport exquisitely express the dynamic structural interactions of lipids and proteins in membrane organization, but our knowledge of the mechanisms involved is not very detailed. However, temperature undoubtedly plays a crucial role in membrane organization. For instance, membrane lipids may form at least partly fluid domains in the membrane core (see *e.g.* refs 1–9). This may allow a relatively rapid diffusion of functional components within the membranes (*e.g.* refs 10–15). However, lowering

the temperature generates lipid phase transitions probably from a liquid–crystalline state to a crystalline phase as shown indirectly by calorimetry[1,16–18] and X-ray diffraction[5] in membranes of different organisms. Such phase transitions are strongly influenced by the fatty acid composition: the larger the proportion of unsaturated fatty acids, the lower the transition point (*e.g.* refs 19–21). It is of particular interest in this connection, that poikilothermic organisms as well as metazoa can lower their lipid transition points by increasing the proportion of unsaturated fatty acids at lower temperatures of growth[1,19,22–35].

Since the fatty acids of membrane lipids seem to be exclusively located in the hydrophobic membrane core, chilling should induce structural changes in this membrane region. Indeed, we have previously shown drastic alterations in the cores of the alveolar membranes in the poikilothermic eukaryote *Tetrahymena pyriformis*, by freeze–etch electron microscopy[13] which makes these membrane cores visible[36,37]. However, there is no published correlation between fatty acid composition and the freeze–etch image of membranes at different temperatures and we now present such data for the ciliate protozoan *Tetrahymena pyriformis* GL.

MATERIAL AND METHODS

Cultures

12-l cultures of *Tetrahymena pyriformis*, amicronucleate strain GL, were grown axenically in the logarithmic growth phase in proteose peptone–liver medium (2% proteose peptone and 0.1% liver extract) at 28 °C, the optimum growth temperature. The generation time of the cells is about 3 h. The cultures were quickly chilled to 10 °C in an ice–salt mixture and left at this temperature for 24 h. Samples were taken under sterile conditions 0, 2, 4, 8, 16 and 24 h after chilling to 10 °C. An additional sample for freeze-etch electron microscopy was taken at 28 °C just before chilling the culture. Cell counts were performed in a Fuchs-Rosenthal chamber.

Freeze–etch electron microscopy

Tetrahymena cells were fixed for about 10 min in proteose peptone–liver medium with 2.5% glutaraldehyde buffered with 5 mM Tris–HCl–10 mM magnesium acetate (pH 6.8). The cells were then washed with this medium and incubated in graded solutions of glycerol up to 25% (buffered with the same solution) for 2–3 h. The cells were concentrated by low-speed centrifugation and frozen on 3-mm copper discs in liquid Freon 22. Fracturing, etching and replicating was carried out using a Balzers machine; etching was done for 1 min at −100 °C[38]. The replicas were viewed in a Siemens Elmiscope 1A.

Fatty acid analysis

Cells were fixed with 3% formaldehyde, washed first with 5 mM Tris–HCl–10 mM magnesium acetate (pH 6.8), then five times with 5% trichloroacetic acid, and subsequently twice with distilled water at 0–5 °C. Total lipids were extracted with chloroform–methanol (2:1) according to the method of Folch *et al.*[39]. Fatty acids were analyzed as methyl esters after alkaline hydrolysis by gas–liquid chromatography on 10% EGSSX on 100–120 mesh gas-chrom P as previously described[40].

The methyl esters were identified before and after catalytic hydrogenation on platinum oxide by comparison with reference substances purchased from Applied Science Laboratories.

RESULTS

Cultures

Chilling log-phase *Tetrahymena* cells to 10 °C drastically inhibited cell division for about 16 h (Fig. 1). After this period, division could again be observed, *i.e.* the *Tetrahymena* cells can adapt to low temperature (*cf.* ref. 41).

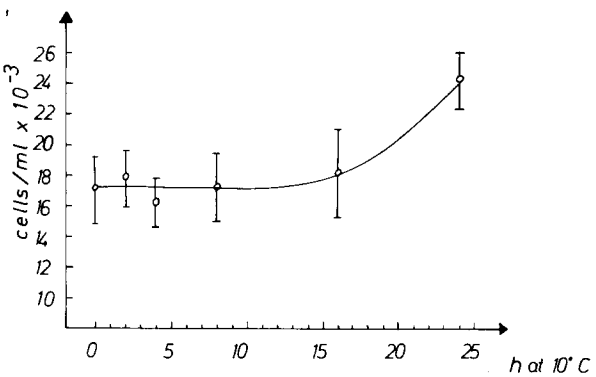

Fig. 1. Growth curve of *Tetrahymena* cells first grown to mid-log phase at 28 °C and then chilled to 10 °C. Cells adapted to the low temperature about 16 h after chilling to 10 °C.

Freeze–etch electronmicroscopy

Cleavage of frozen *Tetrahymena* membranes resulted in their splitting, exposing their apolar core and exposing two faces per membrane type with distinct freeze–etch patterns. The face patterns of the fractured membranes of mitochondria, peroxisomes, mucocysts, vacuoles and smaller vesicles from cells chilled to 10 °C resembled those from cells grown at 28 °C before chilling, though occasional structural alterations were observed (*cf.* ref. 42). However, cooling always produced drastic alterations in the membrane cores of the pellicula, alveolar sacs, endoplasmic reticulum and nuclei. We therefore concentrated upon the structural description of these membrane-types.

Cells grown at 28 °C before chilling to 10 °C

The inner faces of the fractured pellicula membrane were studded with a lot of randomly distributed 70-Å particles (*cf.* Fig. 3), while the outer faces showed only a few particles. In contrast, the outer faces of the fractured alveolar membranes revealed randomly distributed 115-Å particles (*cf.* Fig. 5). These obviously penetrate the whole alveolar membrane and thus leave corresponding holes on the inner faces as described previously[13,42]. The outer faces of the fractured endoplasmic reticulum membranes were covered with a lot of randomly distributed particles, whereas only a few particles (*cf.* Fig. 8) but relatively more holes were revealed on the inner faces (*cf.* Fig. 8).

Comparable patterns were also observed on the inner and outer fracture faces

of nuclear membranes (*cf.* Fig. 11). The nuclear membranes fused together focally to form the characteristic nucleopores. Very often, focal fusions were also seen in the endoplasmic reticulum membranes, disrupting the endoplasmic reticulum cisternae.

Cells chilled to 10 °C

After cooling the inner faces of the fractured pellicula membranes not only showed the typical particle regions but also large depressed and smooth areas with a few 70-Å particles (Fig. 2). We believe that these smooth depressions are sites where the pellicula membranes have come into contact with the underlying outer alveolar membranes. The 115-Å particles and the corresponding holes on the outer and inner

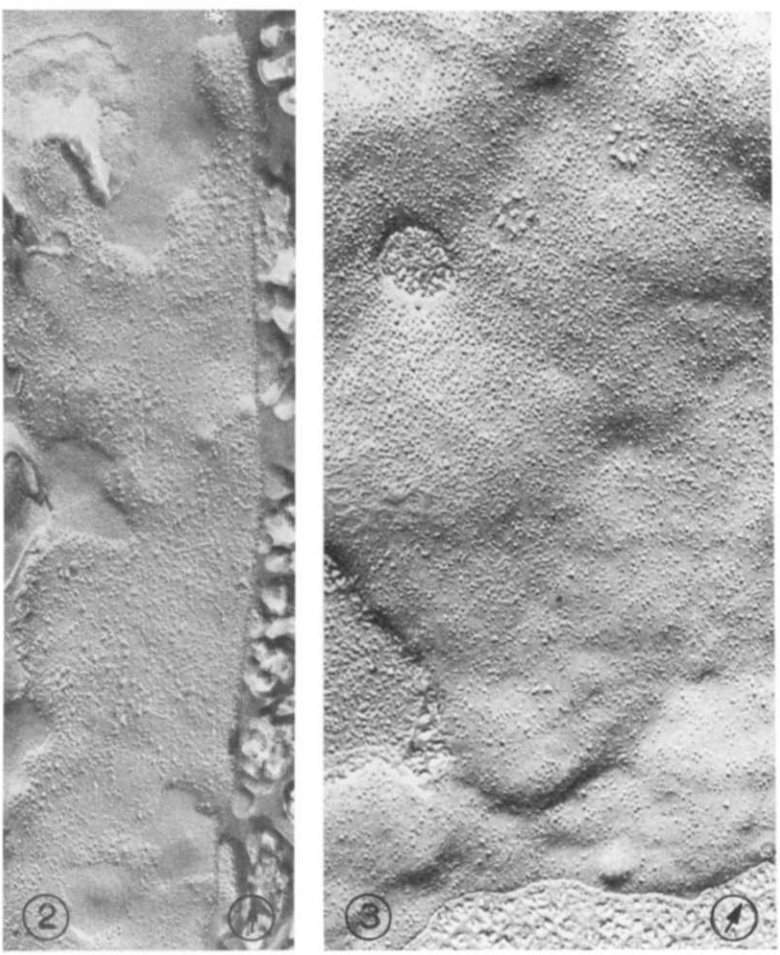

Fig. 2. Freeze-etch image of a cell chilled to 10 °C. Smooth depressions are observed on the inner faces of the fractured pellicula membrane. × 80000.

Fig. 3. Cell at 16 h after chilling to 10 °C. Particles on the inner faces of the fractured pellicula membrane are again randomly distributed. In the upper part, the aperture of a discharged mucocyst and the cartwheel structure of the preformed opening sites of a mucocyst can be observed. × 80000.

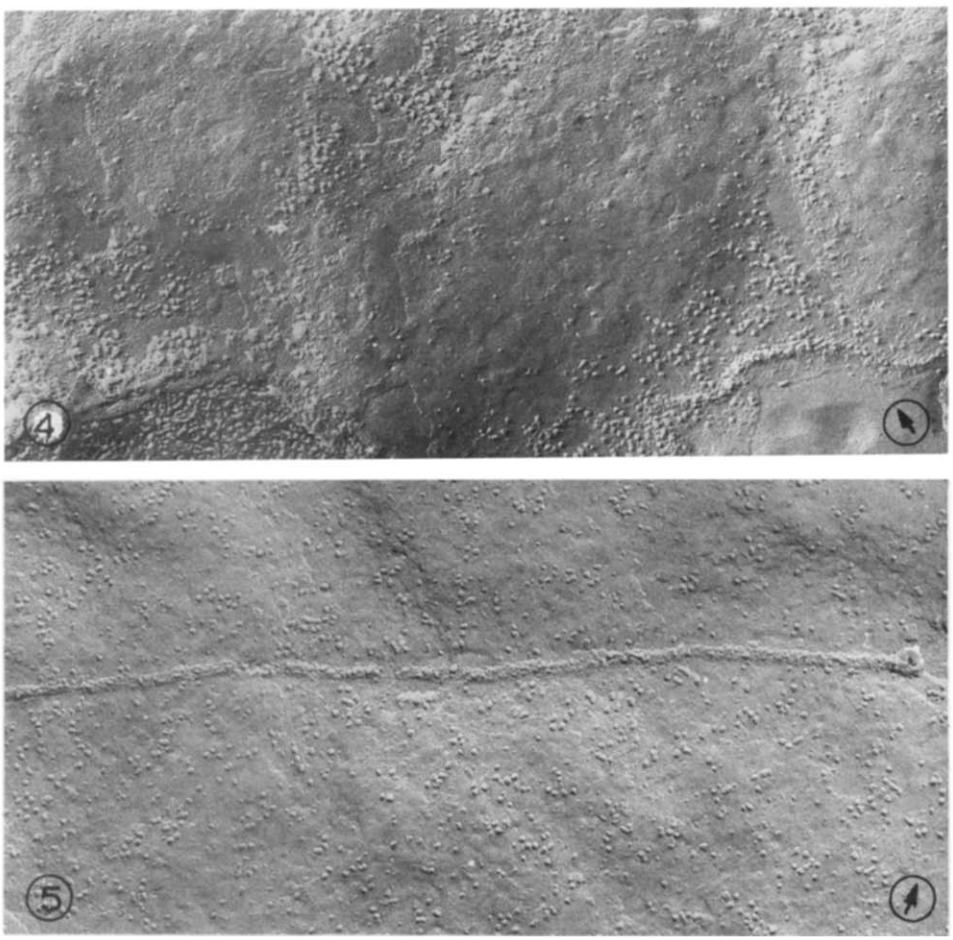

Fig. 4. Cell chilled to 10 °C. The 115-Å particles are aggregated on the outer faces of the fractured alveolar membranes. × 80000.

Fig. 5. Cell at 16 h after chilling to 10 °C. Particles are again randomly distributed on the outer faces of the fractured alveolar membranes of two adjacent alveolar sacs. × 80000.

faces of the fractured alveolar membranes were largely aggregated (Fig. 4). Moreover. this aggregation could be still enhanced upon slowly chilling the cells within about 4 min as shown previously[13]. The inner and outer faces of the fractured membranes of the endoplasmic reticulum and the nuclei also revealed large smooth areas (Figs 6, 7, 9, 10). In addition, the number of disruptions in the endoplasmic reticulum cisternae appeared to be less extensive. These findings indicate that temperature lowering induces drastic and quick reorientation of membrane components. The low temperature patterns were still seen 2, 4 and 8 h after chilling the cells to 10 °C. However, 8 h after chilling, about 50% of the face patterns of the endoplasmic reticulum and nuclear membranes recovered the appearance seen in cells grown at 28 °C (Fig. 8).

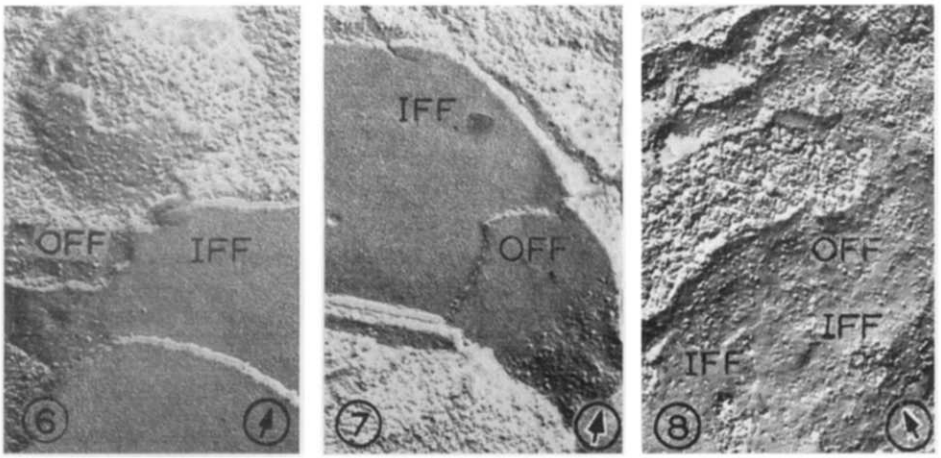

Fig. 6. Cell chilled to 10 °C. Randomly distributed particles on the outer face (OFF) and large smooth areas on the inner face (IFF) of the fractured membranes of an endoplasmic reticulum cisterna. Below, the smooth face of the fractured membrane of an adjacent endoplasmic reticulum. × 80000.

Fig. 7. Cell chilled to 10 °C. The outer face of the fractured membranes (OFF) of an endoplasmic reticulum cisterna as well as the inner face (IFF) of the fractured membranes of an adjacent endoplasmic reticulum cisterna reveal smooth areas. × 80000.

Fig. 8. Cell at 16 h after chilling to 10 °C. Both inner (IFF) and outer faces (OFF) of endoplasmic reticulum membranes are again randomly studded with particles and holes. × 80000.

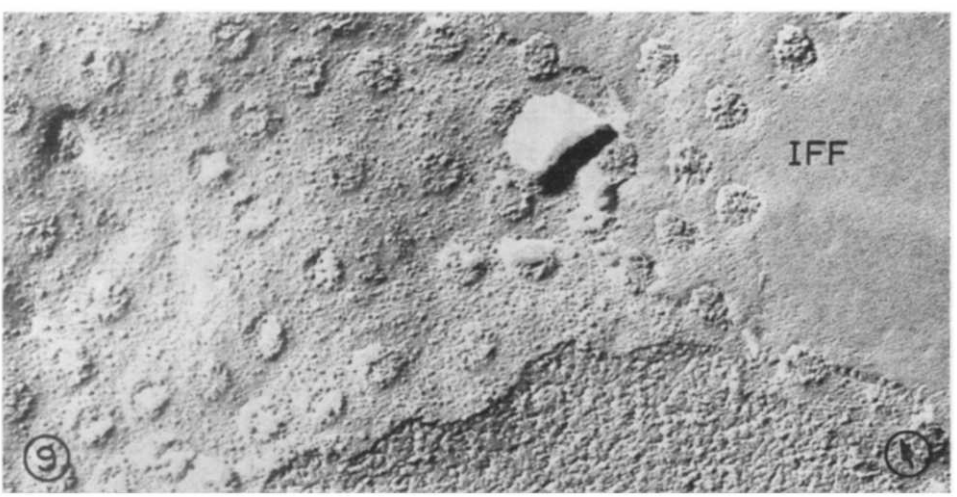

Fig. 9. Cell chilled to 10 °C. The inner faces of the outer nuclear membranes reveal smooth areas without nucleopores. × 80000.

About 16 h after chilling to 10 °C the random distribution of membrane-intercalated particles of all the *Tetrahymena* membranes was restored to that obtained in 28 °C controls (Figs 3, 5, 8, 11), though the quality of the fracture faces of different replicas varied slightly (*cf.* ref. 43).

Fig. 10. Cell chilled to 10 °C. The outer faces (OFF) of the inner fractured nuclear membrane shows smooth areas without nucleopores. × 80000.

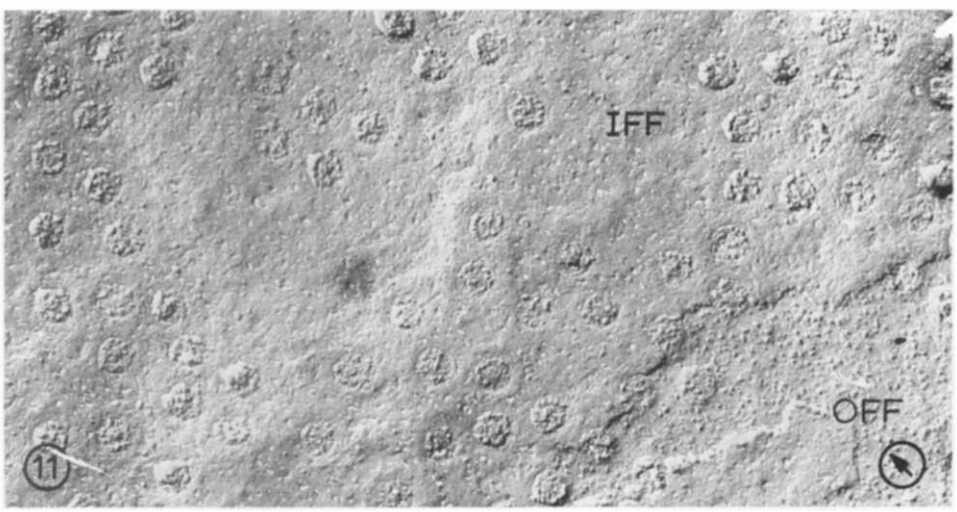

Fig. 11. Cell at 16 h after chilling to 10 °C. Both inner (IFF) and outer faces (OFF) of the fractured outer and inner nuclear membranes now lack smooth areas. × 80000.

Fatty acid analysis

The total lipids of *Tetrahymena* cells contained essentially even-numbered fatty acids ranging from C_{12} to C_{20} (Table I), the main constituents being C_{16} and C_{18} fatty acids. Appreciable amounts of C_{20} acids were also detected.

At the beginning of exposure to low temperature the cells contained about 25% saturated fatty acids. However, 16 h later this amount was reduced to about

13%, indicating that during the period of inhibited cell division the *Tetrahymena* cells replaced about 50% of the saturated fatty acids with unsaturated acids (Table I, Fig. 12).

TABLE I

FATTY ACID COMPOSITION OF LIPIDS FROM *TETRAHYMENA PYRIFORMIS* GL AT VARIOUS TIME INTERVALS AFTER CHILLING TO 10 °C (% OF TOTAL FATTY ACIDS)

Acid	*0 h*	*2 h*	*4 h*	*8 h*	*16 h*	*24 h*
12:0	1.7	1.2	1.5	1.1	1.0	1.1
14:0	7.9	7.1	7.4	7.5	5.0	5.7
16:0	8.6	7.1	6.4	5.2	4.4	3.3
16:1	7.3	7.2	7.7	8.1	11.2	13.8
18:0	2.5	1.3	1.2	1.1	0.9	0.9
18:1	9.0	10.5	8.8	10.8	13.0	14.0
18:2	28.9	28.9	29.4	28.5	27.5	24.9
18:3	26.2	28.9	30.2	31.1	30.6	30.4
20:0	3.2	3.0	2.7	2.2	2.1	2.2
20:2	0.2	0.2	0.3	0.4	0.5	0.5
20:3	3.4	3.5	3.3	2.9	2.6	2.1
Unknown	1.1	1.1	1.1	1.0	1.2	1.1
Saturated	23.9	19.7	19.2	17.1	13.4	13.2
Unsaturated	75.0	79.2	79.7	81.8	85.4	85.7
Amount of double bonds	163	172	176	179	180	176

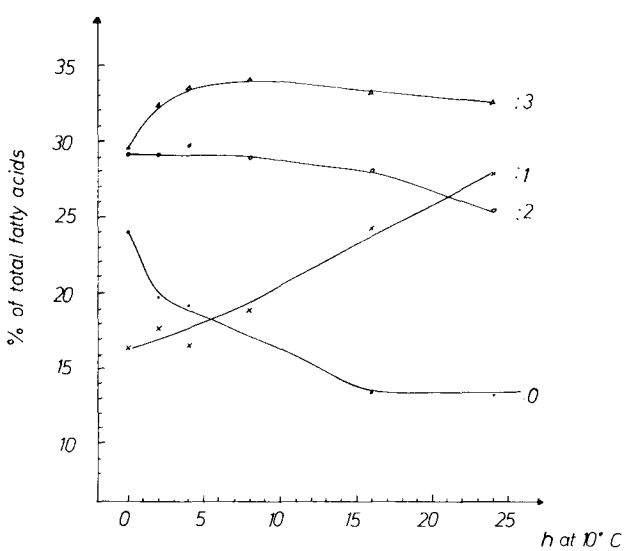

Fig. 12. Proportion of saturated fatty acids, and monoenoic, dienoic, and trienoic acids of *Tetrahymena* cells chilled to 10 °C.

The unsaturated fatty acid fraction of *Tetrahymena* was mainly composed of monoenoic, dienoic, and trienoic acids. The unsaturated acid pattern changed during the 24-h period at 10 °C (Fig. 12). Among the individual unsaturated acids, the monoenoic acids revealed the most dramatic alterations, increasing almost linearly from about 16–28% during the low-temperature period. In contrast, the amount of dienoic acids remained nearly constant for the first 8 h, and then decreased slightly. The trienoic acids increased markedly during the first 4 h after chilling; subsequently the proportion of these fatty acids remained constant at about 33%. In this connection, it is of interest that the absolute number of double bonds in the total fatty acid fraction increased during the first 8 h at 10 °C (Table I). Further incubation at low temperature did not alter this number though changes in the fatty acid pattern were still considerable (Table I, Fig. 12).

DISCUSSION

We have shown by freeze–etch electron microscopy that chilling *Tetrahymena* cells to 10 °C causes drastic structural alterations in the apolar membrane cores of the pellicula, the alveolar sacs, endoplasmic reticulum, and nuclei; the face pattern of these fractured membrane types reveal more particle-free areas after cooling. Previously we have shown, however, still larger particle-free areas: Upon cooling *Tetrahymena* cells to 5 °C, particle-free areas always begin to emerge in the alveolar membrane cores at about 15 °C, enlarge at 10 °C and dilate still more at 5 °C[13]. This phenomenon of increase of smooth-faced areas at decreasing temperatures also holds true for the membrane cores of the pellicula, endoplasmic reticulum and nuclei (Wunderlich, F. and Speth, V., unpublished).

Smooth-faced areas appear to be mainly lipid domains of the membrane core[13]. The membrane-intercalating particles, however, apparently represent at least partly proteins and/or glycoproteins complexed with lipids[11,41,45]. Thus, we have previously suggested that this low temperature-induced segregation of membrane core components is primarily due to crystallization in the membrane lipid phase with the two-dimensionally growing lipid crystals displacing the "proteic" particles[13].

This view is in accord with recent data of Blazyk and Steim[17], who found using scanning calorimetry, that rat liver microsomes and microsomal lipids reveal thermotropic phase transitions ranging between about −10 °C and 10 °C, centered at 0 °C. Moreover, our findings suggest that the higher the degree of crystallization of membrane lipids, the larger is the segregation of membrane core components in *Tetrahymena* and *vice versa*.

Furthermore, our results show that the segregation of membrane core components at 10 °C is not lethal for *Tetrahymena* cells. The cells are capable to revert these low-temperature-induced alterations of the membrane cores, *i.e.* the original membrane structure is restored before the *Tetrahymena* cells can divide again at low temperature. In close correlation, the structural recovery of membranes is accompanied by a desaturation of about 50% of the saturated fatty acids in the total cell lipid fraction. It is interesting that the most marked alterations in the fatty acid composition occur during the first 8 h after chilling and are essentially completed when the cells have adapted to the low temperature, although subsequently some minor changes are still found (Table I, Fig. 12). This is in accord with the findings

of other authors (*cf.* refs 19, 22–35) who reported that at lower growth temperatures the proportion of unsaturated fatty acids increases in different organisms. The fatty acids are incorporated mainly into the phospholipids and make up an essential part of the apolar membrane core. In *Tetrahymena* only traces of free fatty acids are detectable (ref. 46; and Kleinig, H., unpublished results) and these may be still a product of lipolytic enzymes, the activities of which are rather strong in *Tetrahymena*[46].

We tend to conclude, therefore, that the structural recovery of the original membrane state in *Tetrahymena* cells at low temperatures is primarily effected by a substitution of unsaturated fatty acids for saturated fatty acids in the membrane phospholipids. In consequence, this would mean that those lipid domains of the membrane cores which crystallized at 10 °C are again fluid. Under those conditions the membrane-associated particles recover their random distribution which seems to be necessary for the optimal functioning of membrane transport processes[11,13].

It appears that certain eukaryotic cells can adjust the "fluidity" of some of their membranes by what must be a refined-temperature controlled system of phospholipid metabolism. However, the mechanisms involved are unknown. We cannot decide whether in our case this is due to a simple exchange of the organisms phospholipids with those in the nutrient medium, to transacylation *in situ* or *de novo* synthesis of phospholipids. Our findings indicate only that the moieties of the individual unsaturated fatty acids in the phospholipids are not so crucial for membrane "fluidity", but rather the ratio between the absolute number of double bonds of the unsaturated phospholipid species and the amount of saturated species per membrane type (*cf.* Table I).

Since our work is still preliminary with respect to the illucidation of this intriguing problem of controlling membrane phospholipid synthesis in eukaryotic cells which might be closely related with membrane genesis, we will focus our future work on the isolation and lipid characterization of the different membrane types in the presented *Tetrahymena* system.

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